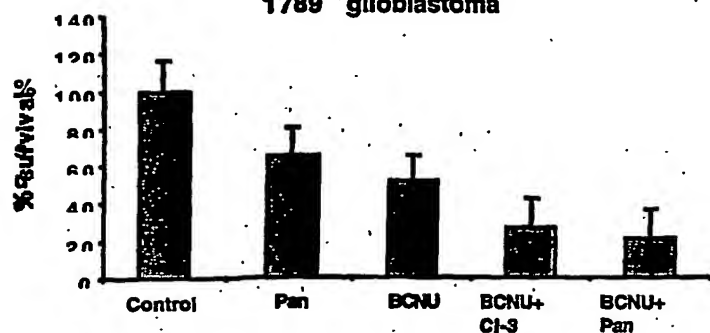


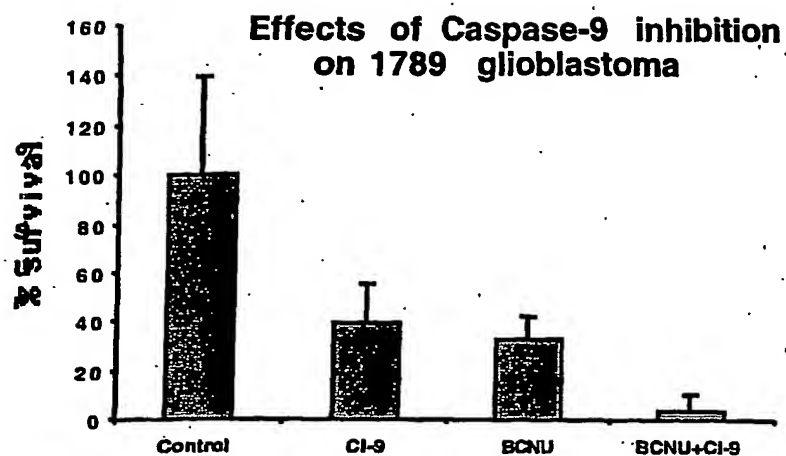
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**Caspase-3-Inhibitor and Pan-Caspase-Inhibitors  
enhance cytotoxicity of BCNU in  
1789 glioblastoma**



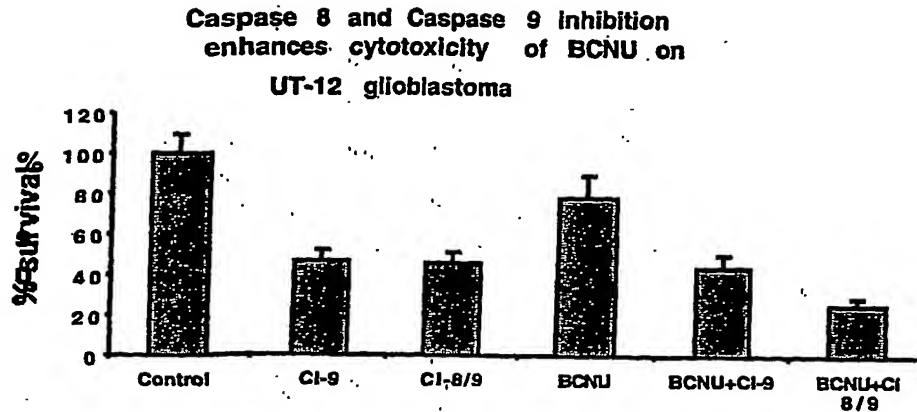
**Figure 1.** Exposure of the 1789 glioblastoma cell line to a pan-caspase inhibitor causes a reduction in cell number equivalent to the effects of exposure to BCNU. Combined exposure to BCNU and the pan-caspase inhibitor significantly increased the amount of cell death over that caused by exposure to BCNU alone. A similar enhancement of BCNU-induced killing was caused by co-exposure to BCNU and an inhibitor of caspase 3.

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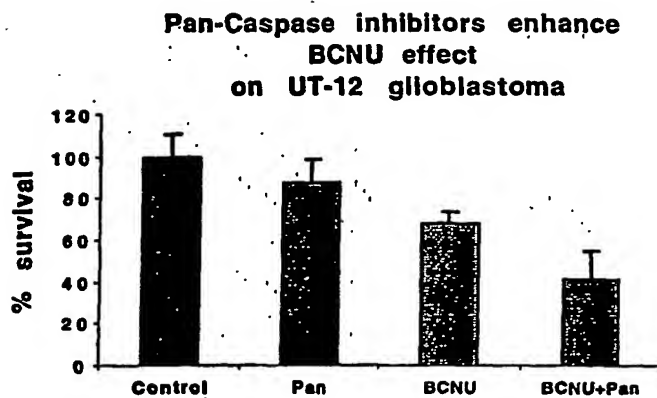
**Figure 2.** Exposure of the 1789 glioblastoma cell line to an inhibitor of caspase-9 causes a reduction in cell number equivalent to the effects of exposure to BCNU. Combined exposure to BCNU and an inhibitor of caspase-9 significantly increased the amount of cell death over that caused by exposure to BCNU alone.

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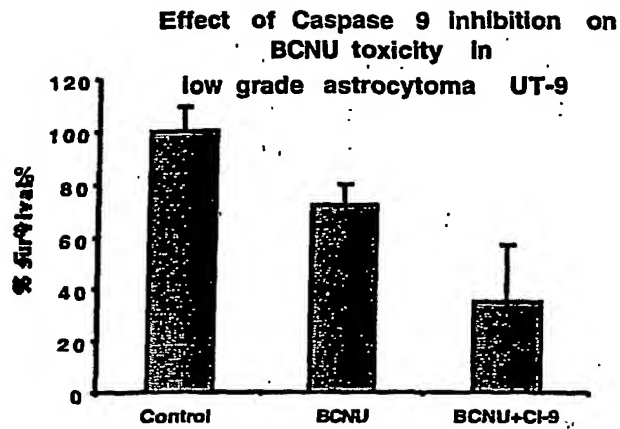
**Figure 3.** Exposure of the UT-12 glioblastoma cell line to an inhibitor of caspase-9 causes a reduction in cell number even greater than the effects of exposure to BCNU. Similar reductions are caused by exposure to a combination of caspase 8 and caspase 9 inhibitors. Combined exposure to BCNU and inhibitors of caspase-8 and caspase-9 applied together with BCNU was associated with significantly increased cell death over that caused by exposure to BCNU alone. In addition, the combination of BCNU and inhibitors of caspase 8 and 8 caused a significantly greater killing of cancer cells than did application of the caspase inhibitors by themselves or by the application of BCNU by itself.

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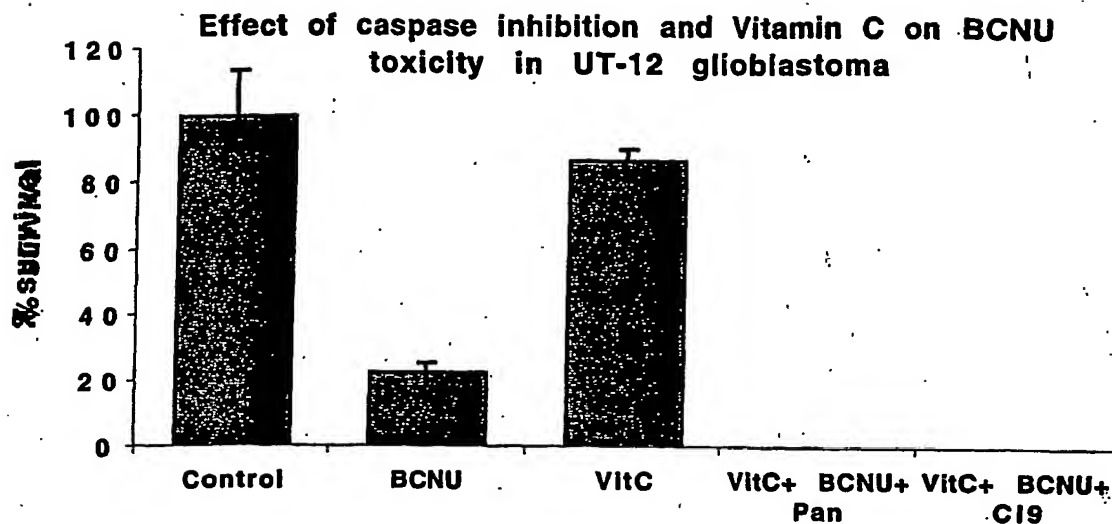
**Figure 4.** Combined exposure of the UT-12 glioblastoma cell line to BCNU and a pan-caspase inhibitor significantly increased the amount of cell death over that caused by exposure to BCNU alone.

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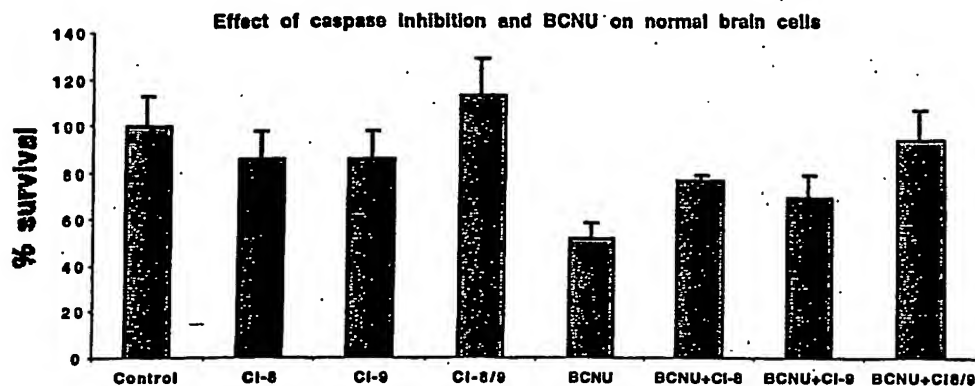
**Figure 5.** Exposure of the UT-9 astrocytoma cell line (derived from a low grade astrocytoma, WHO grade II) to BCNU (at equivalent doses used for the glioblastoma cell lines 1789 and UT-12) causes only a minor reduction in cell number. In contrast, when BCNU is added together with an inhibitor of caspase-9 the number of cells killed is significantly increased. These experiments that caspase inhibitor activation may also be able to overcome chemoresistance.

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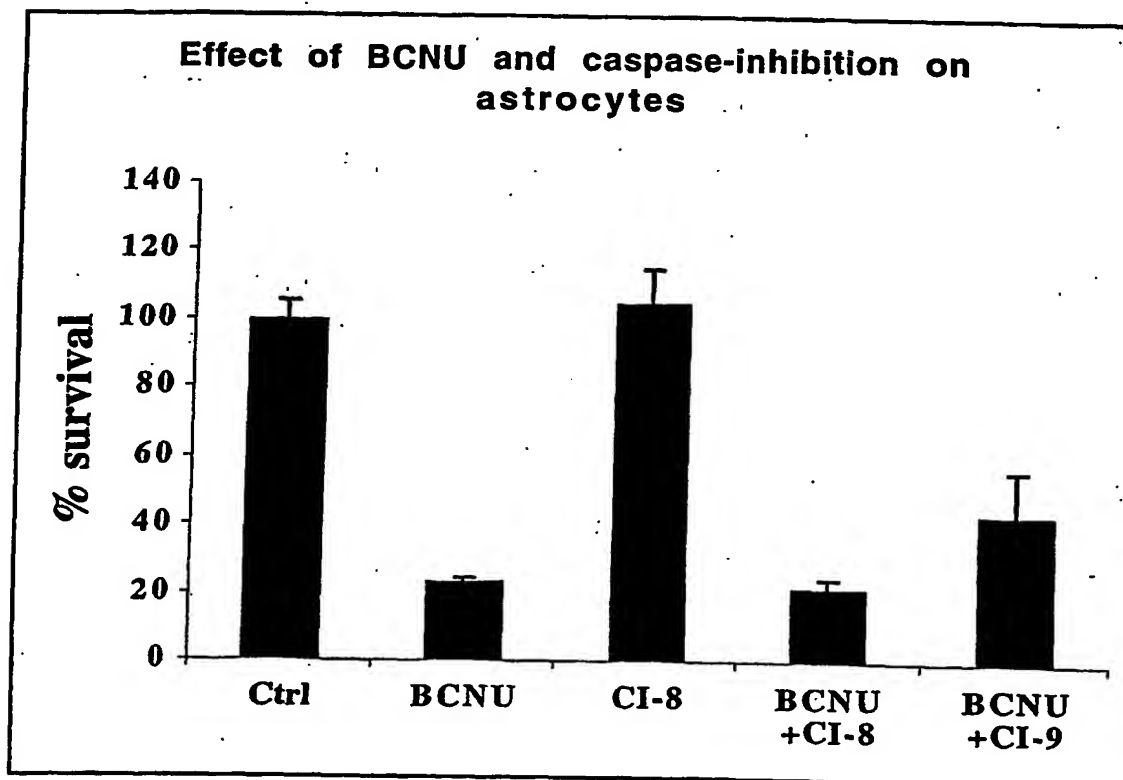
**Figure 6.** These experiments demonstrate that the anti-oxidant Vitamin C fails to rescue tumor cells from death induced by exposure to BCNU and caspase inhibitors. In comparison with Figure 4, one sees that the introduction of the anti-oxidant makes killing of cancer cells even more effective.

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**Effect of caspase inhibitors on normal brain cells:**

**Figure 7.** In contrast to the effects of caspase inhibitors in enhancing the killing of tumor cells (as shown in Fig. 1-5), these same inhibitors do not have such effects on normal human brain precursor cells. This example shows treatment of human glia restricted precursor cells (GRP) with BCNU. Caspase 8 and 9 inhibitors do not enhance the cytotoxic activity of BCNU nor do they compromise the viability of human GRP cells when applied by themselves.

**Figure 8. Caspase inhibitors do not enhance the cytotoxic effects of BCNU on normal astrocytes.** Cells were plated (1000cells/well) on 24-well-coverslips and exposed to BCNU (40 $\mu$ g/ml for 1 h) alone or to BCNU in combination with caspase inhibitors (CI-8 and CI-9). Cells were exposed to caspase inhibitors for 24hrs at a concentration of 20 $\mu$ M each. After a 48hr recovery period, cells were MTT/DAPI-stained to determine viability. Error bars represent s.e.m.

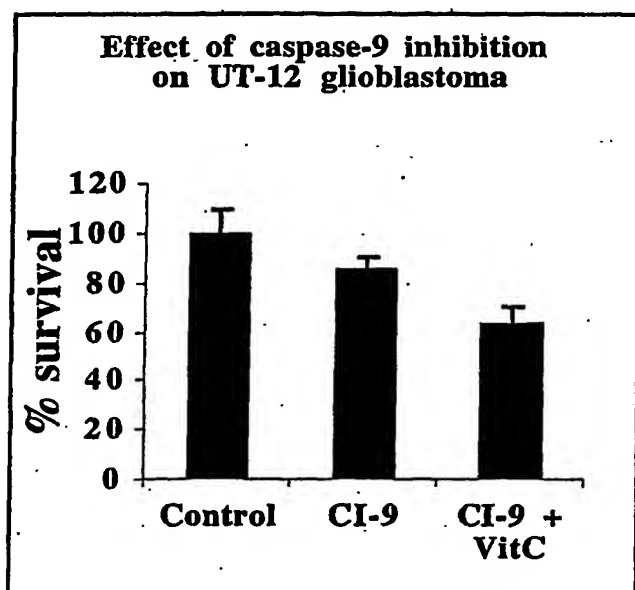




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**Figure 9. Caspase 9 inhibitor and Vitamin C decrease survival of UT-12 glioblastoma cells.**

Cells were plated (1000cells/well) on 24-well-coverslips and exposed to caspase 9 inhibitor (20 $\mu$ M)  $\pm$  Vitamin C (20  $\mu$ g/ml) for 24 hrs. After a 48hrs recovery period, cells were MTT/DAPI-stained to determine viability. Error bars represent s.e.m.



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**Caspase inhibitors do not rescue cisplatin-induced toxicity on SW480 colon cancer cells.**

The figure shows DAPI-staining of SW480 cells after 24hrs treatment with caspase-inhibitors  $\pm$  cisplatin, thus revealing the cellular nuclei.

A. Control. B. Pan-Inhibitor. C. Caspase-3 inhibitor. D. Cisplatin. E. Cisplatin+Pan-Inhibitor. F. Cisplatin+caspase-3 inhibitor. The caspase inhibitors and cisplatin were added at a concentration of 20 $\mu$ M.

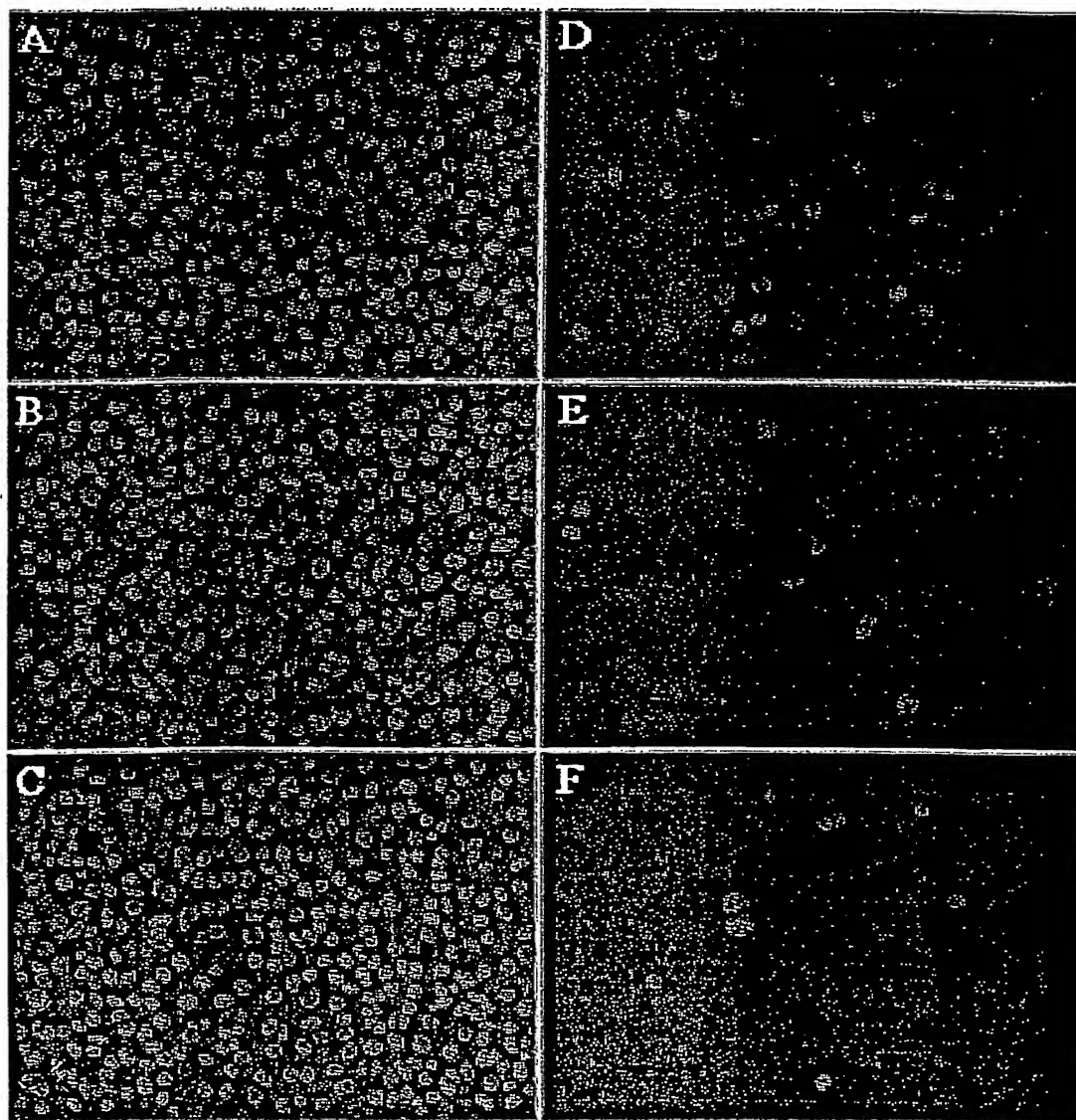


Figure 10

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